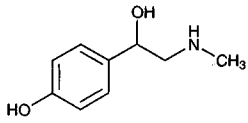
Synephrine



Molecular formula: $C_9H_{13}NO_2$
Molecular weight: 167.21
CAS Registry No.: 94-07-5, 5985-28-4 (HCl), 6414-49-9 (tartaric acid monoester), 16589-24-5 (tartrate)
Merck Index: 9189

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 3.02

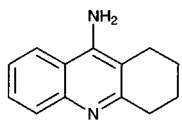
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tacrine



Molecular formula: $C_{13}H_{14}N_2$
Molecular weight: 198.27
CAS Registry No.: 321-64-2, 1684-40-8 (HCl)
Merck Index: 9199
Lednicer No.: 5 166

SAMPLE

Matrix: bile, dialysate
Sample preparation: Bile. 100 µL Bile + 50 µL 500 mM NaOH, vortex for 1 min, add 500 µL ethyl acetate, vortex for 2 min, centrifuge at 12000 g for 30 s. Remove the organic layer and evaporate it to dryness under a stream of argon at 55°, reconstitute the residue in 100 µL

Ringer's solution, inject a 5 μL aliquot. Dialysate. Inject a 5 μL aliquot of dialysate directly. (The dialysis solution was Ringer's solution that contained 155 mM NaCl, 5.5 mM KCl, and 2.3 mM $CaCl_2$.)

HPLC VARIABLES
Column: 100 × 1 5 μm Spherisorb phenyl
Mobile phase: MeCN:MeOH:50 mM pH 2.5 ammonium phosphate buffer 5:10:85
Flow rate: 0.045
Injection volume: 5
Detector: F ex 330 em 365

CHROMATOGRAM
Retention time: 11
Limit of detection: 0.25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
microbore; rat; pharmacokinetics

REFERENCE
Hadwiger,M.E.; Telting-Diaz,M.; Lunte,C.E. Liquid chromatographic determination of tacrine and its metabolites in rat bile microdialysates, *J.Chromatogr.B*, **1994**, *655*, 235–241.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 500 mM NaOH + 5 mL dichloromethane, shake gently for 15 min, centrifuge at 500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 150 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:20 mM pH 2.7 phosphate buffer 20:80
Flow rate: 1.1
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Ekman,L.; Lindström,B.; Roxin,P. Determination of tacrine and its 1-hydroxy metabolite in plasma using column liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1989**, *494*, 397–402.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 250 μL picric acid (1:50 dilution of saturated picric acid solution) + 250 μL water + 2.5 mL dichloromethane:isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150-250 μL MeCN:water 40:60, centrifuge at 1500 g for 4 min, inject a 20-100 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 μPorasil
Mobile phase: MeOH:0.1 mM sulfuric acid 50:50
Flow rate: 2
Injection volume: 20-100
Detector: UV 210

CHROMATOGRAM
Retention time: 5
Internal standard: tacrine

OTHER SUBSTANCES
Simultaneous: laudanosine

KEY WORDS
plasma; tacrine is IS

REFERENCE
Bjorksten,A.R.; Beemer,G.H.; Crankshaw,D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J.Chromatogr.*, **1990**, *533*, 241–247.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 250 ng IS + 500 μL 500 mM NaOH, vortex briefly, add 5 mL cyclohexane:ethyl acetate 50:50, shake for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Hypersil phenyl
Mobile phase: MeCN:20 mM pH 2.75 ammonium formate buffer 70:30
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM
Retention time: 15
Internal standard: N-methoxy-1,2,3,4-tetrahydroacridin-9(10H)-imine (18)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Hsu,R.S.; DiLeo,E.M.; Chesson,S.M. High-performance liquid chromatography for the determination of tacrine and its metabolites in plasma, *J.Chromatogr.*, **1990**, *530*, 170–176.

SAMPLE
Matrix: blood, urine
Sample preparation: Blood. Add 25 μL 10 μM IS , 250 μL 500 mM sodium hydroxide, and 5 mL ethyl acetate to 500 μL plasma, shake for 10 min, centrifuge at 1100 g for 10 min. Keep at -30° until the aqueous phase is frozen. Evaporate the organic phase to dryness under a stream of nitrogen at 40° for 45 min, reconstitute in 300 μL mobile phase, vortex for 30 s, centrifuge at 1100 g for 1 min and at 13000 g for 15 min. Take a 100 μL sample from the middle and inject. Urine. Add 25 μL 100 μM IS, 250 μL 500 mM sodium hydroxide, and 5 mL ethyl acetate to 500 μL plasma, shake for 10 min and centrifuge at 1100 g for 10 min. Keep at -30° until the aqueous phase is frozen. Evaporate the organic phase to dryness under a

stream of nitrogen at 40° for 45 min, reconstitute in 900 μL mobile phase and vortex for 30 s. Inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: LiChrospher 60 RP-select B
Column: 250 × 4 5 μm LiChrospher 60 RP-select B
Mobile phase: MeCN:200 mM pH 4.0 acetate buffer 13:87 (Buffer was 16.406 g sodium acetate in 1 L water, pH adjusted to 4.0 with 60% perchloric acid.)
Column temperature: 30
Flow rate: 1.25 for 16 min, 2.5 for 24 min
Injection volume: 100
Detector: F ex 330 em 365

CHROMATOGRAM
Retention time: 32.93 (blood), 31.71 (urine)
Internal standard: 1,2,3,4,-tetrahydro-9-acridanone (Aldrich) (30.39 (blood), 29.30 (urine))
Limit of detection: 2 nM (blood), 80 nM (urine)
Limit of quantitation: 2 nM (blood), 120 nM (urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Hansen,L.L.; Larsen,J.T.; Brosen,K. Determination of tacrine and its metabolites in human plasma and urine by high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.B*, **1998**, *712*, 183–191.

SAMPLE
Matrix: diffusate, tissue
Sample preparation: Homogenize (Polytron PCU-2) 150-200 mg skin and diazepam with 4 mL chloroform, repeat homogenization, filter (phase-separating paper) extracts. Make the residue alkaline with 2 mL 10% NaOH, extract twice with 4 mL portions of chloroform, wash the extracts twice with 2 mL portions of water, filter (phase-separating paper) the organic layer. Combine all the chloroform layers and evaporate them to dryness under a stream of air, reconstitute the residue in 1 mL mobile phase, filter (microfilter), inject an aliquot.

HPLC VARIABLES
Guard column: 20 × 4 40 μm ODS (Valco)
Column: 150 × 4.6 5 μm Spherisorb ODS-I
Mobile phase: MeCN:water 52:48 containing 10 mM octanesulfonic acid and 1% acetic acid, pH 3.5
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.7
Internal standard: diazepam (6.0)
Limit of detection: 5 μg/g

OTHER SUBSTANCES
Extracted: physostigmine

KEY WORDS
skin; pharmacokinetics; stability-indicating

REFERENCE

Lau,S.W.J.; Chow,D.; Feldman,S. Simultaneous determination of physostigmine and tetrahydroaminoacridine in a transdermal permeation study by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *526*, 87–95.

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Microsomal incubation + 5 mL ice-cold MeCN, let stand overnight at 4°, centrifuge at 1000 g for 15 min. Evaporate the supernatant to dryness, reconstitute in MeOH, inject an aliquot.

HPLC VARIABLES

Guard column: present but not specified
Column: 250 mm long Ultracarb 5 C8 (Phenomenex)
Mobile phase: MeCN:100 mM pH 3.9 ammonium acetate buffer 12:88
Flow rate: 1.25
Detector: UV 254

CHROMATOGRAM

Retention time: 18

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; rat; mouse; dog; hamster; rabbit; liver

REFERENCE

Madden,S.; Spaldin,V.; Hayes,R.N.; Woolf,T.F.; Pool,W.F.; Park,B.K. Species variation in the bioactivation of tacrine by hepatic microsomes, *Xenobiotica*, **1995**, *25*, 103–116.

SAMPLE

Matrix: microsomal incubations

Sample preparation: 1 mL Microsomal incubation + 5 mL MeCN, let stand at 4° overnight, centrifuge at 1000 g. Remove the supernatant and evaporate it to dryness, reconstitute the residue in a small volume of MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.2 Nucleosil 5 C8
Mobile phase: Gradient. MeCN:100 mM pH 3.8 ammonium acetate buffer 10:90 for 15 min, to 15:85 over 5 min, to 20:80 over 10 min.
Flow rate: 1.25
Detector: UV 254

CHROMATOGRAM

Retention time: 32

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

human; liver

REFERENCE

Spaldin,V.; Madden,S.; Adams,D.A.; Edwards,R.J.; Davies,D.S.; Park,B.K. Determination of human hepatic cytochrome P4501A2 activity *in vitro*. Use of tacrine as an isoenzyme-specific probe, *Drug Metab.Dispos.*, **1995**, *23*, 929–934.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: urine
Sample preparation: 0.5-1 mL Urine + 1 μg IS + 1 mL 1 M NaOH, vortex briefly, add 5 mL cyclohexane:ethyl acetate 50:50, shake for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL mobile phase, inject an aliquot. (Hydrolyze conjugates by heating 0.5-1 mL urine, 2 mL 100 mM pH 5 acetate buffer, and 1000 units β-glucuronidase (type H-1, Sigma) at 37° for 4 h, proceed as above.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Hypersil phenyl
Mobile phase: MeCN:50 mM ammonium formate buffer 70:30, pH 3.1
Flow rate: 1.5
Detector: UV 325

CHROMATOGRAM
Internal standard: N-methoxy-1,2,3,4-tetrahydroacridin-9(10H)-imine

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; pharmacokinetics

REFERENCE
Hsu,R.S.; Shutske,G.M.; DiLeo,E.M.; Chesson,S.M.; Linville,A.R.; Allen,R.C. Identification of the urinary metabolites of tacrine in the rat, *Drug Metab.Dispos.*, **1990**, *18*, 779–783.

SAMPLE
Matrix: urine
Sample preparation: Evaporate 5 μg of IS dissolved in MeOH into the bottom of a tube using a stream of nitrogen, add 1 mL urine, add 1 mL water, add 500 μL 5 M NaOH, add 5 mL chloroform, shake, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL mobile phase, inject the whole amount. (To hydrolyze conjugates heat 1 mL urine containing 5 μg IS and 1 mL enzyme solution at 37° for 16 h, add 500 μL 5 M NaOH, proceed as above. Prepare enzyme solution by diluting 400 μL 100000 U/mL β-glucuronidase and 3000 U/mL sulfatase (Type H2, Sigma) to 20 mL with 100 mM pH 5.0 acetate buffer.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: Hexane:isopropanol:diethylamine 70:30:0.1
Flow rate: 1
Injection volume: 100
Detector: UV 254

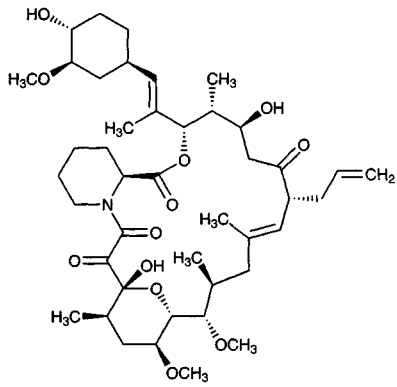
CHROMATOGRAM
Retention time: 27
Internal standard: 1-amino-4-nitronaphthalene (9)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Hooper,W.D.; Pool,W.F.; Woolf,T.F.; Gal,J. Stereoselective hydroxylation of tacrine in rats and humans, *Drug Metab.Dispos.*, **1994**, *22*, 719–724.

Tacrolimus

Molecular formula: $C_{44}H_{69}NO_{12}$
Molecular weight: 804.03
CAS Registry No.: 104987-11-3
Merck Index: 9200



SAMPLE

Matrix: blood
Sample preparation: Add 100 µL 50 ng/mL IS in MeOH + 1.0 mL water + 5 mL dichloromethane:cyclohexane 40:60 to 500 µL whole blood. Mix using reciprocating shaker at low speed for 1 hour, centrifuge at 3000 rpm for 10 min. Remove the organic phase (leaving at least 0.5 cm behind) and evaporate it to dryness. Reconstitute the residue with 50 µL MeOH. Inject a 5 µL aliquot.

HPLC VARIABLES

Column: 100 × 2.1 5 µm ODS-Hypersil
Mobile phase: MeOH:5 mM ammonium acetate adjusted to pH 9.0 with 28-30% ammonium hydroxide solution 99:1
Flow rate: 0.3
Injection volume: 5
Detector: MS, PE SCIEX (Perkin-Elmer Sciex) API III MS/MS, nebulizer 400° interface 60°, auxiliary flow (nitrogen) 6.0 L/min, nebulizer flow (nitrogen) 0.6 L/min, curtain gas (nitrogen) 1.2 L/min, collision energy 40 V, (m/z 802.5)

CHROMATOGRAM

Retention time: 0.97
Internal standard: FR900520 (Fujisawa Pharmaceutical, Japan) (0.95) (m/z 790.6)
Limit of detection: 100 pg/mL

KEY WORDS

whole blood; pharmacokinetics

REFERENCE

Alak,A.M.; Moy,S.; Cook,M.; Lizak,P.; Niggebiugge,A.; Menard,S.; Chilton,A. An HPLC/MS/MS assay for tacrolimus in patient blood samples. Correlation with results of an ELISA assay, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 7–13.

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL blood containing IS with and MeCN:MeOH:zinc sulfate 20:30:50, mix, centrifuge. Add the supernatant to a C18 SPE cartridge. Evaporate the eluate to dryness at 40°. Reconstitute the residue with 100 µL MeCN:water 50:50. Inject a 75 µL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 3 µm Hypersil BCS CPS
Mobile phase: MeCN:water 50:50
Column temperature: 68
Flow rate: 1
Injection volume: 75
Detector: MS, tandem mass, negative API mode, m/z 802.4-560.5

CHROMATOGRAM
Limit of quantitation: 25 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE
Hill,H.M.; Clarke,S.D.; Bentley,L.; Noctor,T.A.G.; Iwasaki,K.; Shiraga,T.; Hata,T.; Undre,N. A high sensitivity assay for tacrolimus (FK506) in human blood (Abstract 2125), *Pharm.Res.*, **1997**, *14*, S261.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 500 μL 2% saponin in water, vortex for 10 s, after 5 min add 2 mL 180 mM HCl and 6 mL diethyl ether, shake at 60 rpm for 15 rpm, centrifuge at 4000 rpm for 10 min. Remove the organic layer and add it to 2 mL 95 mM NaOH, shake at 60 rpm for 15 min, centrifuge at 4000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL MeCN, vortex for 1 min, add 100 μL 1% trifluoroacetic acid in MeCN, add 100 μL 0.6 mg/mL dansylhydrazine in MeCN, vortex for 1 min, evaporate to dryness under a stream of nitrogen at 37°, store in the dark at 4°. Just before analysis reconstitute in 100 μL MeCN, inject a 25 μL aliquot onto column A with mobile phase A, elute column A with mobile phase A for 3 min, after 3 min elute contents of column A onto column B with mobile phase B, after 30 s remove column A from circuit, elute column B with mobile phase B for 6.5 min, elute tacrolimus fraction with mobile phase B from column B onto column C for 2 min, elute column C with mobile phase C and monitor the effluent. (Flush column A with MeOH for 5 min then re-equilibrate with mobile phase A for 4 min before next injection.)

HPLC VARIABLES
Column: A 15 × 3.9 25-40 μm C18 (Applied Biosystems); B 150 × 3.9 4 μm Novapack C18; C 100 × 3.2 Hypercarb Ph graphite
Mobile phase: A MeOH:water 50:50; B MeCN:water 80:20; C MeOH:dichloromethane 50:50
Flow rate: A 1; B 1; C 1.5
Injection volume: 25
Detector: F ex 338 em 520 (or 430 nm cut-off filter)

CHROMATOGRAM
Retention time: 3.5 (after the start of the elution of column C with mobile phase C)
Limit of quantitation: 3 ng/mL

KEY WORDS
whole blood; column-switching; heart-cut

REFERENCE
Beysens,A.J.; Beuman,G.H.; van der Heijden,J.J.; Hoogtanders,K.E.J.; Steijger,O.M.; Lingeman,H. Determination of tacrolimus (FK 506) in whole blood using liquid chromatography and fluorescence detection, *Chromatographia*, **1994**, *39*, 490–496.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Whole blood + 400 μL MeCN, agitate for 15 min, centrifuge at 9500 g for 5 min. Evaporate the supernatant to dryness at 40°, reconstitute with 70 μL isopropanol, agitate for 30 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 mm long 3 μm Spherisorb CN
Mobile phase: Hexane:isopropanol 72:28
Column temperature: 40
Flow rate: 1.8

Injection volume: 50
Detector: Immunoassay (Evaporate fraction eluting between 4.1 and 4.9 min, reconstitute with 100 μL drug-free whole blood, treat with 200 μL reagent (Abbott IMx Tacrolimus).)

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites, acetaminophen, N-acetylprocainamide, amikacin, azathioprine, carbamazepine, cyclosporine, digitoxin, digoxin, diltiazem, disopyramide, erythromycin, ethosuximide, flecainide, gentamicin, lidocaine, methylprednisolone, netilmicin, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
whole blood

REFERENCE
Firdaous,I.; Hassoun,A.; Otte,J.B.; Reding,R.; Squifflet,J.P.; Besse,T.; Wallemacq,P.E. HPLC-microparticle enzyme immunoassay specific for tacrolimus in whole blood of hepatic and renal transplant patients, *Clin.Chem.*, **1995**, *41*, 1292–1296.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 5 mL ethyl acetate, shake for 30 min, centrifuge at 1660 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL THF, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 TSKgel ODS-80TM (TOSOH)
Mobile phase: MeCN:water 60:40
Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 220

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
whole blood

REFERENCE
Namiki,Y.; Fujiwara,A.; Kihara,N.; Koda,S.; Hane,K.; Yasuda,T. Determination of the immunosuppressive drug tacrolimus in its dosage forms by high-performance liquid chromatography, *Chromatographia*, **1995**, *40*, 253–258.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 3 mL Recipe C18 SPE cartridge with 2 mL MeCN and 2 mL water adjusted to pH 3.0 with sulfuric acid. 1 mL Blood or urine + 10 μL 1 μg/mL IS in MeCN:water 70:30 (pH 3.0) + 2 mL MeOH:1 M zinc sulfate 70:30, vortex for 20 s, centrifuge at 2000 g for 2 min, add the supernatant to the SPE cartridge, wash with 2 mL water, dry cartridge, elute with 400 μL MeCN:pH 3.0 water 90:10, inject a 99 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Spherical C18 (Waters)
Mobile phase: MeOH:water 90:10
Flow rate: 0.4
Injection volume: 99

Detector: MS, Hewlett-Packard 5989 A quadrupole, type 59980 B particle-beam interface, chemical ionization, reagent gas methane, electron multiplier 2646 V, particle-beam interface 55°, ionization source 250°, quadrupole 100

CHROMATOGRAM
Retention time: 5.2
Internal standard: 32-O-acetyltacrolimus (6.4)
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE
Gonschior,A.-K.; Christians,U.; Winkler,M.; Schiebel,H.M.; Sewing,K.-F. Simplified high-performance liquid chromatography-mass spectrometry assay for measurement of tacrolimus and its metabolites and cross-validation with microparticle enzyme immunoassay, *Ther.Drug Monit.*, **1995**, *17*, 504–510.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Dissolve 25 mg tacrolimus in 300 μg/mL heptyl p-hydroxybenzoate in EtOH + 25 mL water, let stand for 6 h at room temperature, inject a 10 μL aliquot. Capsules. Extract 10 capsules with 300 μg/mL heptyl p-hydroxybenzoate in EtOH, centrifuge at 1660 g for 10 min, dilute an aliquot of the supernatant with an equal volume of water, let stand for 6 h at room temperature, inject a 10 μL aliquot. Ampules. 2.5 mL Ampule solution + 22.5 mL 300 μg/mL heptyl p-hydroxybenzoate in EtOH + 25 mL water, let stand for 6 h at room temperature, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 TSKgel ODS-80TM (TOSOH)
Mobile phase: THF:isopropanol:water 2:2:5
Column temperature: 50
Flow rate: 0.8
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 10
Internal standard: heptyl p-hydroxybenzoate (14.5)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
ampules; capsules

REFERENCE
Namiki,Y.; Fujiwara,A.; Kihara,N.; Koda,S.; Hane,K.; Yasuda,T. Determination of the immunosuppressive drug tacrolimus in its dosage forms by high-performance liquid chromatography, *Chromatographia*, **1995**, *40*, 253–258.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 60:40 containing 2 mM sodium 1-hexanesulfonate

Column temperature: 70
Flow rate: 1.5
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 12.6

OTHER SUBSTANCES
Simultaneous: cimetidine

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Ku,Y.-M.; Min,D.I.; Kumar,V.; Noormohamed,S.E. Compatibility of tacrolimus injection with cimetidine hydrochloride injection in 0.9% sodium chloride injection, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2024–2025.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 50 μL 1 M HCl + 3.5 mL ethyl acetate, shake for 10 min. Remove 3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen below 40°, reconstitute the residue in 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 Inertsil ODS-2
Column: 250 × 4.6 Inertsil ODS-2
Mobile phase: Gradient. MeCN:0.1% phosphoric acid from 36:64 to 54:46 over 20 min, to 100:0 over 7 min, return to initial conditions over 2.1 min
Column temperature: 50
Flow rate: from 1.2 to 2.5 over 27.1 min
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 28.4

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat

REFERENCE
Iwasaki,K.; Shiraga,T.; Matsuda,H.; Nagase,K.; Tokuma,Y.; Hata,T.; Fujii,Y.; Sakuma,S.; Fujitsu,T.; Fujikawa,A.; Shimatani,K.; Sato,A.; Fujioka,M. Further metabolism of FK506 (Tacrolimus). Identification and biological activities of the metabolites oxidized at multiple sites of FK506, *Drug Metab.Dispos.*, **1995**, *23*, 28–34.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 3 mL glass 25-40 μm C8 SPE cartridge with 3 mL MeCN and 3 mL pH 3.0 sulfuric acid. 1.5 mL Microsomal incubation + 500 μL MeCN, mix, centrifuge at 2500 g for 2 min, add the supernatant to the SPE cartridge, wash with 3 mL MeOH:pH 3.0 sulfuric acid 50:50, wash with 500 μL hexane, pull air through the cartridge for 3 min, elute with 1.5 mL dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 56°, reconstitute with 300 μL MeCN:pH 3.0 sulfuric acid 70:30, wash this solution with 500 μL hexane, inject a 125 μL aliquot of the MeCN layer.

HPLC VARIABLES
Column: 250 × 4 3 μm Hypersil C8

Mobile phase: Gradient. MeCN:pH 3.0 sulfuric acid from 42:58 to 48:52 over 20 min, to 57:43 over 15 min, to 75:25 over 10 min (concave gradient), wash with 95:5 for 5 min, re-equilibrate at initial conditions for 7 min.
Column temperature: 75
Flow rate: 0.7
Injection volume: 125
Detector: UV 205

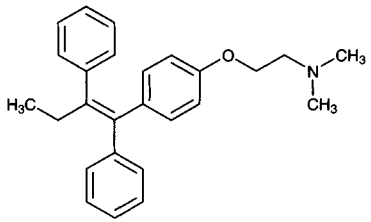
CHROMATOGRAM
Retention time: 26
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; rat; pig; intestines; SPE

REFERENCE
Lampen,A.; Christians,U.; Guengerich,F.P.; Watkins,P.B.; Kolars,J.C.; Bader,A.; Gonschior,A.-K.; Dralle,H.; Hackbarth,I.; Sewing,K.-F. Metabolism of the immunosuppressant tacrolimus in the small intestine: Cytochrome P450, drug interactions, and interindividual variability, *Drug Metab.Dispos.*, **1995**, *23*, 1315–1324.

Tamoxifen



Molecular formula: $C_{26}H_{29}NO$
Molecular weight: 371.52
CAS Registry No.: 10540-29-1, 54965-24-1 (citrate)
Merck Index: 9216
Lednicer No.: 2 127; 3 70; 4 65

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bond-elut C2 SPE cartridge with 2 mL MeOH and 2 mL water. Apply 1 mL plasma to the cartridge, wash with 1 mL water, wash with 1 mL MeOH: water 50:50, wash with 1 mL MeCN, elute with 2 mL 1 M NaCl:MeOH 5:95. Dry the eluate under vacuum, resuspend in 200 μL MeOH, inject a 20 μL aliquot. GC 10 mm long C18 (Waters)

HPLC VARIABLES
Column: 30 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:1% pH 8.0 triethylamine 89:11
Flow rate: 1.2
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 10.45
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma

REFERENCE
MacCallum,J.; Cummings,J.; Dixon,J.M.; Miller,W.R. Solid-phase extraction and high-performance liquid chromatographic determination of tamoxifen and its major metabolites in plasma, *J.Chromatogr.B*, **1996**, *678*, 317–323.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL cyano-bonded silica (J.T.Baker) SPE cartridge with 1 mL MeOH and three 1 mL portions of water. Mix 100 μL serum with 500 μL water. Add to the SPE cartridge and allow to pass through by gravity. Wash three times with 1 mL potions of water and with 1 mL MeOH:water 50:50. Elute with 1 mL MeOH containing 1 mL/L triethylamine. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax cyano bonded silica
Mobile phase: MeCN:MeOH:phosphate buffer 23:37:40, adjusted to pH 3.5
Column temperature: 45
Flow rate: 0.8
Injection volume: 20
Detector: UV 241

CHROMATOGRAM
Retention time: 5.57
Internal standard: tamoxifen

OTHER SUBSTANCES
Extracted: amiodarone, desethylamiodarone, bepridil, L8040 (Sanofi Recherche), trifluoperazine
Simultaneous: aprindine, bromocriptine, captopril, carbamazepine, chlorpromazine, diltiazem, dimefline, dipyridamole, disopyramide, flecainide, flurazepam, furosemide, imipramine, labetalol, miconazole, nifedipine, norverapamil, procainamide, propafenone, propranolol, quinidine, tocainide, trifluoropromazine, verapamil, warfarin

KEY WORDS
tamoxifen is IS; serum; SPE

REFERENCE
Pollak,P.T. A systematic review and critical comparison of internal standards for the routine liquid chromatographic assay of amiodarone and desethylamiodarone, *Ther.Drug Monit.*, **1996**, *18*, 168–178.

SAMPLE
Matrix: blood
Sample preparation: Extract 2 mL serum with two 20 mL portions of diethyl ether, evaporate to dryness using compressed air, reconstitute the residue in 1 mL MeOH, centrifuge at 3000 rpm for 3 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hichrom ODS 1 (Anachem, Luton, UK)
Mobile phase: MeCN:MeOH:water:trichloroacetic acid 31:50:18.9:0.1, adjusted to pH 2.9
Flow rate: 1.8
Injection volume: 20
Detector: F ex 254 em 360 following post-column reaction. The column effluent flowed through a 200 cm × 0.2 mm ID coil of PTFE tubing irradiated by a mercury UV lamp to the detector.

CHROMATOGRAM
Retention time: 8.3 (E), 9.2 (Z)
Limit of detection: 100 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; chiral; post-column photochemical derivatization; post-column reaction

REFERENCE
Manns,J.E.; Hanks,S.; Brown,J.E. Optimised separation of E- and Z-isomers of tamoxifen, and its principal metabolites using reversed-phase high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 847–852.

SAMPLE
Matrix: blood
Sample preparation: Vortex serum with 10 volumes hexane:butanol 98:2 for 15 s, centrifuge for 10 min. Remove an aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 50-100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm ODS-2 (Whatman)
Mobile phase: MeOH containing 0.04% diethylamine acetate
Flow rate: 2
Detector: F ex 220 or 254 em 360 following post-column reaction. The column effluent flowed through a 70 cm × 0.2 mm ID quartz coil irradiated with two Mineralite short-wave UV lamps to the detector.

CHROMATOGRAM
Retention time: 5.4
Limit of detection: 0.2 ng

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
post-column reaction; post-column photochemical derivatization; serum; protect from light

REFERENCE
Brown,R.R.; Bain,R.; Jordan,V.C. Determination of tamoxifen and metabolites in human serum by high-performance liquid chromatography with post-column fluorescence activation, *J.Chromatogr.*, **1983**, *272*, 351–358.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 2 μg clomiphene + 2 mL diethyl ether, extract, centrifuge at 2000 rpm at 4° for 10 min, repeat extraction. Combine the organic phases and evaporate them to dryness under a stream of nitrogen at 37°. Reconstitute the residue in 250 μL MeOH, centrifuge at 2000 rpm at 4° for 10 min, inject a 10-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: MeCN:10 mM KH_2PO_4:300 mM phosphoric acid :water 42:20:10:28
Flow rate: 2.8
Injection volume: 10-100
Detector: F ex 258 em 378 following post-column reaction. The column effluent flowed through a 6.5 m × 0.35 mm × 1.5 mm o.d. PTFE tube irradiated with a Philips HPK 125 watt high pressure mercury lamp to the detector.

CHROMATOGRAM
Retention time: 9
Internal standard: clomiphene (12)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; post-column photochemical derivatization

REFERENCE
Milano,G.; Etienne,M.C.; Frenay,M.; Khater,R.; Formento,J.L.; Renee,N.; Moll,J.L.; Francoual,M.; Berto,M.; Namer,M. Optimised analysis of tamoxifen and its main metabolites in the plasma and cytosol of mammary tumours, *Br.J.Cancer*, **1987**, *55*, 509–512.

SAMPLE
Matrix: blood
Sample preparation: 800 μL Plasma + 200 μL 100 mM HCl, centrifuge at 12000 g for 2 min. Inject the following solutions onto column A; 500 μL MeOH, 700 μL water, 300 μL 100 mM HCl, 500 μL supernatant, and 1 mL water. Flush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 35 × 2 40 μm Sepralyte CN-propyl modified silica (Analytichem); B 110 × 4.6 Partisil Si
Mobile phase: MeOH:5 mM ammonium acetate 90:10
Flow rate: 1.4
Injection volume: 500
Detector: F ex 256 em 380 following post-column reaction. The column effluent flowed through a 15 m × 0.25 mm ID crocheted PTFE tube irradiated with a Sylvania G8 UV lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 3
Limit of detection: 100 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; post-column photochemical derivatization; column-switching

REFERENCE
Kikuta,C.; Schmid,R. Specific high-performance liquid chromatographic analysis of tamoxifen and its major metabolites by "on-line" extraction and post-column photochemical reaction, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 329–337.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL phenyl SPE cartridge with five 1 mL portions of MeCN, 1 mL water, and 1 mL 2.5 mL/L triethylamine in 50 mM pH 3.0 phosphate buffer. 980 μL Plasma + 30 μL MeOH:water 50:50 + 1 mL MeCN, vortex for 1 min, centrifuge at -10° at 2500 g for 1 h. Remove a 1.6 mL aliquot of the supernatant and add it to 400 μL 2% heptanesulfonic acid in 50 mM pH 3.0 KH_2PO_4/phosphoric acid buffer, add to the SPE cartridge, wash with two 100 μL portions of MeCN:buffer 80:20, wash with 50 μL 25 mM sulfuric acid, elute with five 100 μL portions of MeCN:buffer 80:20. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL MeCN:buffer 70:30, inject a 10-30 μL aliquot. (Buffer was 5 mM heptanesulfonic acid in 50 mM pH 3.0 phosphate buffer.)

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Si-100-S Phenyl (BST, Budapest)
Column: 250 × 4.6 10 μm Si-100-S Phenyl (BST, Budapest)
Mobile phase: MeCN:buffer 75:25 (Buffer was 50 mM pH 3.0 KH_2PO_4/phosphoric acid buffer containing 5 mM heptanesulfonic acid and 300 μL/L triethylamine. Temperature of MeCN was 60° and temperature of buffer was 80°.)
Flow rate: 1.2
Injection volume: 10-30

Detector: F ex 257 em 378 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID knitted PTFE coil irradiated by a mercury lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 7.03
Internal standard: tamoxifen

OTHER SUBSTANCES
Extracted: panomifene

KEY WORDS
post-column reaction; post-column photochemical derivatization; plasma; pharmacokinetics; SPE; tamoxifen is IS

REFERENCE
Erdélyi-Tóth,V.; Pap,E.; Kralovánszky,J.; Bojti,E.; Klebovich,I. Determination of panomifene in human plasma by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *668*, 419–425.

SAMPLE
Matrix: blood
Sample preparation: 150 μL Plasma + 150 μL MeCN, vortex for 2 min, centrifuge at 13000 g for 5 min, inject 50 μL supernatant onto column A with mobile phase A, elute column A to waste for 2 min with mobile phase A, elute column A to waste for another 2 min with mobile phase B, elute column A onto column B with mobile phase B, analyze effluent from column B. (Single pump used. Switch from mobile phase A to mobile phase B by switching solvent reservoirs.)

HPLC VARIABLES
Column: A SPS CN guard column (Regis); B Regis C18 guard column + 250 × 4.6 5 μm Regis Rexchrom CN
Mobile phase: A water; B MeCN: 20 mM pH 3.1 K_2HPO_4 35:65
Flow rate: A 1; B 1
Injection volume: 50
Detector: F ex 250 em 370 (cut-off filter) preceded by a photochemical reactor, ICT Beam Boost, 254 nm UV lamp, 5 m reaction coil.

CHROMATOGRAM
Retention time: 47
Limit of detection: 8 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, 4-hydroxytamoxifen, N-desdimethyltamoxifen, N-desmethyltamoxifen, tamoxifen-ol

KEY WORDS
plasma; rugged; post-column photochemical derivatization

REFERENCE
Fried,K.M.; Wainer,I.W. Direct determination of tamoxifen and its four major metabolites in plasma using coupled column high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *655*, 261–268.

SAMPLE
Matrix: blood
Sample preparation: Extract 2 mL plasma twice with 3 mL portions of hexane:EtOH 98:2. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeCN, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 LC-18 (Supelco)
Mobile phase: Gradient. MeCN:20 mM ammonium acetate 20:80 for 4 min, to 40:60 over 20 min, to 65:35 over 16 min

Flow rate: 0.75
Injection volume: 10-20
Detector: UV 280 or MS, Finnigan TSQ triple quadrupole, electrospray, atmospheric pressure ionization, capillary 220°, electrode -4.5 kV, drying gas nitrogen, 50% of column effluent directed into MS, SIM 372

CHROMATOGRAM
Retention time: 42.33

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; LC-MS

REFERENCE
Poon,G.K.; Walter,B.; Lonning,P.E.; Horton,M.N.; McCague,R. Identification of tamoxifen metabolites in human Hep G2 cell line, human liver homogenate, and patients on long-term therapy for breast cancer, *Drug Metab.Dispos.*, **1995**, *23*, 377–382.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 50% urea + 5 mL diethyl ether, extract. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:67 mM pH 2.2 phosphate buffer 50:50
Flow rate: 1
Injection volume: 50
Detector: F ex 260 em 375 following post-column reaction. The column effluent flowed though a 7 m × 0.3 mm ID knitted PTFE coil irradiated by a Sylvana G8T6 UV lamp to the detector.

CHROMATOGRAM
Limit of quantitation: 0.51 ng/mL

KEY WORDS
post-column reaction; post-column photochemical derivatization; pharmacokinetics; plasma

REFERENCE
Fuchs,W.S.; Leary,W.P.; Van der Meer,M.J.; Gay,S.; Witschital,K.; von Nieciecki,A. Pharmacokinetics and bioavailability of tamoxifen in postmenopausal healthy women, *Arzneimittelforschung*, **1996**, *46*, 418–422.

SAMPLE
Matrix: blood
Sample preparation: 30 μL Plasma + 12 μL 15 μg/mL quinine bisulfate in MeOH, mix, make up to 90 μL with 600 mM orthophosphoric acid in MeCN, illuminate in a shortwave UV transilluminator (UVP, San Gabriel CA) with 0.25 J/min for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: CN Resolve (Waters)
Column: 100 × 8 10 μm Radial Pak CN radial compression (Waters)
Mobile phase: MeOH:buffer 70:30 (Buffer was 100 mM sodium acetate containing 1 mM tetrabutylammonium phosphate, adjusted to pH 6 with orthophosphoric acid.)
Flow rate: 4
Injection volume: 50
Detector: F ex 258 em 378

CHROMATOGRAM
Retention time: 10.13

Internal standard: quinine bisulfate (4.36)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, aspirin, azathioprine, carmustine, chlorambucil, cytarabine, dacarbazine, N-desmethyltamoxifen, etoposide, 4-hydroxytamoxifen, indomethacin, lomustine, methotrexate, procarbazine, salicylic acid, tenoposide, thioguanine
Noninterfering: cyclophosphamide, doxorubicin, ifosfamide, mitomycin C, prednisone, taxol, vincristine

KEY WORDS
derivatization; plasma

REFERENCE
el-Yazigi,A.; Legayada,E. Direct liquid chromatographic micro-measurement of tamoxifen in plasma of cancer patients, *J.Chromatogr.B*, **1997**, *691*, 457–462.

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. 300 μL Serum + 10 μL 10 μg/mL enclomiphene in EtOH + 1 mL hexane:amyl alcohol 98:2, mix vigorously for 5 min, freeze aqueous layer in dry ice/acetone, remove organic layer, repeat extraction two more times. Combine the extracts and evaporate them to dryness, resuspend the residue in 100 μL mobile phase, inject an aliquot. Tissue. 10 mg Liver or 15 mg uteri or 20 mg muscle + enclomiphene + 1.5 mL MeOH:acetic acid 98:2, homogenize in a 7.5 mL Potter-Elvehjem ground glass-ground glass homogenizer, centrifuge at 2000 g. Remove the organic layer and evaporate it to dryness. Extract the resulting residue with 1 mL acetone with vigorous mixing for 5 min. Centrifuge at 2000 g, remove the acetone extract and evaporate it to dryness, resuspend in 1 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm silica (Scientific Glass Engineering)
Mobile phase: Isooctane:EtOH:isopropanol:diethylamine:acetic acid 75:23.5:1.5:0.05:0.05
Flow rate: 1
Detector: F ex 257 em 383 preceded by a post-column in-line UV reactor

CHROMATOGRAM
Retention time: 3
Internal standard: enclomiphene (2)
Limit of detection: 0.7 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
serum; liver; muscle; uterus; human; rat; mouse; normal phase; post-column photochemical derivatization

REFERENCE
Robinson,S.P.; Langan-Fahey,S.M.; Johnson,D.A.; Jordan,V.C. Metabolites, pharmacodynamics, and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient, *Drug Metab.Dispos.*, **1991**, *19*, 36–43.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 20.652

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 5 mL chilled chloroform to microsomal mixture, vortex, adjust aqueous phase to pH 9, extract with 5 mL chloroform. Combine the organic phases and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeOH:water 85:15, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-Select-B C8
Mobile phase: MeOH:water:triethylamine 80:20:0.01
Flow rate: 0.8
Detector: UV 238, or UV 277, or F ex 258 em 318 preceded by an on-line Knauer UV photoreactor

CHROMATOGRAM
Retention time: 35

OTHER SUBSTANCES
Simultaneous: metabolites
Also analyzed: toremifene

KEY WORDS
post-column photochemical derivatization

REFERENCE
Berthou,F.; Dréano,Y. High-performance liquid chromatographic analysis of tamoxifen, toremifene and their major human metabolites, *J.Chromatogr.*, **1993**, *616*, 117–127.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 2 mL Microsomal incubation + 4 mL MeOH:DMSO 80:20, vortex, centrifuge at 2000 g for 20 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Res Elute-BD (Varian)

Mobile phase: MeOH:500 mM ammonium acetate 70:30
Flow rate: 1
Injection volume: 100
Detector: UV 280, MS, VG Quattro BQ tandem quadrupole, API electrospray, capillary 380 V, high voltage electrode 3.78 kV, source 150°, lens 1 and lens 2 80-85 V, positive ion mode, column flow split 1:6 before entering MS, m/z 372

CHROMATOGRAM
Retention time: 51
Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; liver

REFERENCE
Jones,R.M.; Yuan,Z.-X.; Lamb,J.H.; Lim,C.K. On-line high-performance liquid chromatographic-electrospray ionization mass spectrometric method for the study of tamoxifen metabolism, *J.Chromatogr.A*, **1996**, *722*, 249–255.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic

acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 3 mL Bond-Elut C2 SPE cartridge with 2 mL MeOH and 2 mL water. Dismembrate (Mikro-dismembrator, Braun, Germany) 50 mg tissue in liquid nitrogen, re-suspend in 1 mL DMSO. Centrifuge at 3000 rpm for 10 min. Add the supernatant to the SPE cartridge, wash with 1 mL water, 1 mL MeOH:water, and 1 mL MeCN. Elute with 1 mL MeOH:1 M NaCl 95:5. Dry eluate under a stream of nitrogen, reconstitute with 400 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 mm C18 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:1% pH 9.0 triethylamine 89:11
Flow rate: 1.2
Injection volume: 20
Detector: UV 265

CHROMATOGRAM

Retention time: 11.00
Limit of detection: 20 ng/g

OTHER SUBSTANCES

Extracted: metabolites, cis-tamoxifen

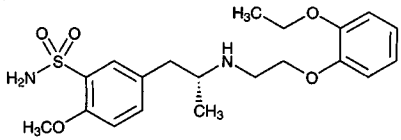
KEY WORDS

breast; tumor; SPE

REFERENCE

MacCallum,J.; Cummings,J.; Dixon,J.M.; Miller,W.R. Solid-phase extraction and high-performance liquid chromatographic determination of tamoxifen and its major metabolites in breast tumour tissues, *J.Chromatogr.B*, **1997**, *698*, 269–275.

Tamsulosin



Molecular formula: $C_{20}H_{28}N_2O_5S$
Molecular weight: 408.52
CAS Registry No.: 106133-20-4, 80223-99-0 ((±) HCl), 106463-17-6 ((R), HCl), 106463-19-8 ((S) HCl)
Merck Index: 9217

SAMPLE

Matrix: bile, microsomal incubations, urine
Sample preparation: Bile, urine. Inject a 50-200 μL aliquot bile or urine. Microsomal incubations. Mix 1 mL microsomal incubation with 5 mL ethyl acetate, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.0 5 μm Nucleosil C18
Mobile phase: Gradient. A was 100 mM pH 4.5 KH_2PO_4. B was MeCN:100 mM pH 4.9 KH_2PO_4 20:80. A:B 100:0 for 5 min, to 0:100 over 60 min, maintain at 0:100 for 60 min
Column temperature: 27
Flow rate: 1
Detector: UV 275; Radioactivity

CHROMATOGRAM

Retention time: 80

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; dog; liver; kidney; intestine; radiolabeled

REFERENCE

Soeishi,Y.; Matsushima,H.; Teraya,Y.; Watanabe,T.; Higuchi,S.; Kaniwa,H. Metabolism of tamsulodin in rat and dog, *Xenobiotica*, **1996**, *26*, 355–365.

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL saturated sodium bicarbonate solution and 100 μL 2.5 μg/mL IS in water to 1.5 mL plasma, extract with 5 mL ethyl acetate. Remove the organic layer and add it to 2.5 mL 400 mM HCl. Shake, centrifuge, and discard the organic layer. Add 2 mL saturated sodium bicarbonate solution to the aqueous layer and extract again with 5 mL ethyl acetate. Evaporate the organic layer to dryness under reduced pressure, reconstitute the residue with 50 μL 100 mM sodium bicarbonate. Add 100 μL 5 mg/mL dansyl chloride in acetone. Heat at 35° for 90 min. Add 5 mL distilled water and extract with 5 mL diethyl ether. Evaporate the organic layer to dryness at 45°, reconstitute the residue with 60 μL mobile phase. Inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 Nucleosil SI100-5 (Chemco, Japan)
Mobile phase: MeOH:benzene 1:100 (Caution! Benzene is a carcinogen!)
Injection volume: 20-50
Detector: F ex 352 em 500

CHROMATOGRAM

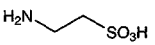
Internal standard: amosulalol
Limit of quantitation: 500 pg/mL (human), 1.0 ng/mL (rat, dog)

KEY WORDS

plasma; human; rat; dog; pharmacokinetics; normal phase; derivatization

REFERENCE
Matsushima,H.; Kamimura,H.; Soeishi,Y.; Watanabe,T.; Higuchi,S.; Tsunoo,M. Pharmacokinetics and plasma protein binding of tamsulosin hydrochloride in rats, dogs, and humans, *Drug Metab.Dispos.*, **1998**, *26*, 240–245.

Taurine



Molecular formula: $C_2H_7NO_3S$
Molecular weight: 125.15
CAS Registry No.: 107-35-7
Merck Index: 9241

SAMPLE
Matrix: amniotic fluid
Sample preparation: 200 μL Amniotic fluid + 800 μL MeOH, mix, centrifuge. 200 μL Supernatant + 80 μL pH 9.5 sodium borate + 60 μL reagent, mix, let stand for 3.5 min, add 25 μL 0.5 M HCl, mix, dilute 1:4 with 50 mM pH 7.0 sodium acetate buffer, inject a 20 μL aliquot. (Prepare reagent by dissolving 50 mg o-phthaldialdehyde in 4.5 mL MeOH, add 500 μL pH 9.5 sodium borate, add 50 μL 2-mercaptoethanol.)

HPLC VARIABLES
Guard column: 10-20 × 4 C18
Column: 300 × 3.9 5 μm NovaPak C18
Mobile phase: Gradient. MeOH:50 mM pH 7.0 sodium acetate buffer from 15:85 to 20:80 over 30 min, to 35:65 over 15 min, to 75:25 over 25 min, maintain at 75:25 for 5 min, return to initial conditions over 5 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 330 em 450

CHROMATOGRAM
Retention time: 50

OTHER SUBSTANCES
Extracted: alanine, 2-aminoadipic acid, 2-aminobutyric acid, 3-aminobutyric acid, 4-aminobutyric acid, arginine, asparagine, aspartic acid, citruline, glutamine, glutathione, glutatic acid, glycine, histidine, homoserine, 5-hydroxylysine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, saccharopine, serine, threonine, tryptophan, tyrosine, valine

KEY WORDS
derivatization

REFERENCE
Klein,B.H.; Dudenhausen,J.W. Ion-exchange chromatography and ion-pair chromatography. Complementation of HPLC analysis of amino acids in body fluids by pre-column derivatization using ortho-phthaldialdehyde, *J.Liq.Chromatogr.*, **1995**, *18*, 4007–4028.

SAMPLE
Matrix: amniotic fluid, blood, CSF, urine
Sample preparation: Plasma. Condition a 100 mg Bond Elut SCX (propylbenzenesulfonic acid, H^+ form) SPE cartridge with 1 mL 50 mM HCl, 1 mL MeOH, 2 mL water, and 1 mL 50 mM HCl. 100 μL Plasma + 100 μL 250 μM norleucine in 100 mM HCl + 10 mg solid sulfosalicylic acid + 800 μL acetone or MeOH, mix, centrifuge, add a 50 μL aliquot to the SPE cartridge, wash with 2 mL water, elute with two 500 μL portions of MeOH:water:triethylamine 40:40:20, dry the eluate under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness

under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Dried blood. Add 25 μL 250 μM norleucine in 100 mM HCl to a 6 mm filter paper disc containing dried blood, add 100 μL MeCN, let stand for 30 min, centrifuge, remove a 75 μL aliquot of the supernatant, evaporate to dryness under reduced pressure, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 2 min, evaporate to dryness under vacuum, reconstitute with 50 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Amniotic fluid, CSF. Mix amniotic fluid or CSF with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 50 (CSF) or 100 (amniotic fluid) μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Urine. Dilute urine with water to a creatinine concentration of 1 mM, mix an aliquot with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 Pico-Tag amino acid column (Waters)
Mobile phase: Gradient. A was MeCN:70 mM pH 6.55 sodium acetate 2.5:97.5. B was MeCN:MeOH:water 45:15:40. A:B 100:0 for 13.5 min, to 97:3 (step gradient), to 94:6 over 10.5 min (Waters curve 8 (slightly concave)), to 91:9 over 6 min (Waters curve 5 (slightly convex)), to 66:34 over 20 min, maintain at 66:34 for 12 min, to 0:100 over 0.5 min, maintain at 0:100 for 4 min, return to initial conditions over 0.5 min.
Column temperature: 46
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 13.82
Internal standard: norleucine (55.07)

OTHER SUBSTANCES

Extracted: α-alanine, alanine, alloisoleucine, β-aminoadipic acid, 4-aminobenzoic acid, gamma-aminobutyric acid, β-amino-n-butyric acid, gamma-amino-n-butyric acid, 4-aminohippuric acid, β-aminoisobutyric acid, 4-aminophenylacetic acid, α-aminophenylacetic acid, 3-amino-3-phenylpropionic acid, δ-amino-n-valeric acid, ammonia, anserine, arginine, asparagine, aspartic acid, aspartylglucosamine, carnosine, citrulline, cystathionine, cysteic acid, cysteine, cysteine-homocysteine (mixed disulfide), cystine, ethanolamine, ethionine, ethylamine, galactosamine, glucosamine, glutamic acid, glutamine, glutathionine (oxidized), glycine, glycylglycine, glycylhistidine, glycylleucine, glycylphenylalanine, glycyltyrosine, histidine, homoarginine, homocitrulline, homoserine, homocystine, 3-hydroxyanthranilic acid, 3-hydroxykynurenine, hydroxyproline, isoleucine, kynurenine, leucine, levodopa, lysine, methionine sulfone, methionine, 3-methylhistidine, 1-methylhistidine, ornithine, phenylalanine, phosphoethanolamine, phosphoserine, proline, sarcosine, serine, serotonin, threonine, tromethamine, tryptophan, tyrosine, valine
Noninterfering: cadaverine, 2-phenylethylamine

KEY WORDS

derivatization; SPE; ultrafiltrate; plasma; dried blood

REFERENCE

Davey,J.F.; Ersser,R.S. Amino acid analysis of physiological fluids by high-performance liquid chromatography with phenylisothiocyanate derivatization and comparison with ion-exchange chromatography, *J.Chromatogr.*, **1990**, *528*, 9–23.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with an equal volume of 5% trichloroacetic acid, centrifuge at 12000 rpm for 5 min. Evaporate the supernatant to dryness, reconstitute with 2-methoxyethanol:0.15 N pH 2.65 sodium citrate 7:93, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4 ISC-07/S1504 Li type (strongly acidic cation-exchange resin of styrene-divinylbenzene copolymer with 10% crosslinkage) (Shimadzu)
Mobile phase: Gradient. A was 2-methoxyethanol:0.15 N pH 2.65 sodium citrate 7:93. B was 0.3 N pH 10.0 sodium citrate. C was 200 mM NaOH. A:B:C from 100:0:0 to 95:5:0 over 50 min, to 85:15:0 over 20 min, to 75:25:0 over 10 min, to 55:45:0 (step gradient), maintain at 55:45:0 for 15 min, to 50:50:0 over 10 min, to 40:60:0 (step gradient), to 30:70:0 over 20 min, to 10:90:0 (step gradient), maintain at 10:90:0 for 15 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 5 min. (Parameters are approximate.)
Column temperature: 38° for 40 min, 52° for 65 min, 55° for 20 min, 58° for 10 min, 38° for 50 min
Flow rate: 0.4
Detector: F ex 348 em 450 following post-column reaction. The column effluent mixed with the reagent solution pumped at 0.2 mL/min and the mixture flowed through a 200 × 0.5 stainless steel or PTFE coil at 55°. The effluent from the coil mixed with the fluorescence solution pumped at 0.2 mL/min and flowed through a 2 m × 0.5 mm stainless steel or PTFE coil at 55° to the detector. (Prepare reagent solution by adding 400 μL NaOCl solution (chlorine concentration 10%) to 1 L buffer, discard after 2 weeks. Prepare fluorescence solution by adding 15 mL EtOH containing 1.6 g o-phthalaldehyde and 2.0 g N-acetyl-L-cysteine and 4 mL 10% Brij 35 in water to 980 mL buffer, discard after 1 month. Buffer contained 384 mM sodium carbonate, 216 mM boric acid, and 108 mM potassium sulfate, pH 10.0.)

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 10 pmole

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
post-column reaction; plasma

REFERENCE
Fujiwara,M.; Ishida,Y.; Nimura,N.; Toyama,A.; Kinoshita,T. Postcolumn fluorometric detection system for liquid chromatographic analysis of amino and imino acids using o-phthalaldehyde/N-acetyl-L-cysteine reagent, *Anal.Biochem.*, **1987**, *166*, 72–78.

SAMPLE
Matrix: blood
Sample preparation: Subject whole blood to two freeze-thaw cycles. 50 μL Plasma or 10 μL whole blood + 200 μL MeCN:MeOH:triethylamine:water 25:22:3:50, vortex for 15 s, filter (Centricon-10 10000 MW exclusion filter) while centrifuging at 2677 g for 15 min. Remove a 20 μL aliquot of the ultrafiltrate and add it to 10 μL 10 mg/mL dansyl chloride in MeCN (prepare fresh daily), vortex, let stand in the dark for 30 min, add 10 μL water:ethylamine 96.5:3.5, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 Spheri-5 ODS
Mobile phase: MeOH:water:acetic acid:triethylamine 33:66.5:0.5:0.025, after 6 min purge column with MeOH:water 90:10 for 4 min, re-equilibrate for 5 min
Column temperature: 50
Flow rate: 1.5
Injection volume: 10
Detector: F ex 329 em 530

CHROMATOGRAM
Retention time: 7
Limit of detection: 2.5 ng

KEY WORDS
cat; plasma; derivatization; ultrafiltrate; whole blood

REFERENCE
Amiss,T.J.; Tyczkowska,K.L.; Aucoin,D.P. Analysis of taurine in feline plasma and whole blood by liquid chromatography with fluorimetric detection and confirmation by thermospray mass spectrometry, *J.Chromatogr.*, **1990**, *526*, 375–382.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 5 μL 2.5 M homoserine, make up to 500 μL with 2 μL/mL 2-mercaptoethanol in MeCN, vortex, centrifuge for 4 min (Beckman microfuge). Remove a 40 μL aliquot of the supernatant and add it to 40 μL reagent and 20 μL 3.7% iodoacetic acid in 400 mM pH 9.5 sodium borate buffer, mix, let stand for 1 min, make up to 200 μL with 100 mM pH 4 potassium phosphate buffer, mix, inject a 20 μL aliquot. (Reagent was 50 mg o-phthaldialdehyde in 1 mL MeOH added to 11 mL 400 mM pH 9.5 sodium borate buffer, 50 μL 2-mercaptoethanol, and 10 mg nitrilotriacetic acid. Filter (0.2 μm), store in the dark at 4°, add 20 μL 2-mercaptoethanol each week to maintain the level of this reagent.

HPLC VARIABLES
Guard column: 5 μm LiChrospher 100 RP-18
Column: 150 × 4.6 5 μm Dynamax Microsorb C18 (Rainin)
Mobile phase: Gradient. A was MeOH:100 mM pH 6.8 sodium acetate buffer 95:5. B was MeOH:100 mM pH 6.8 sodium acetate buffer 5:95. A:B from 15:85 to 30:70 over 15.5 min, to 55:45 over 9 min, to 60:40 over 2 min, to 100:0 over 8 min, maintain at 100:0 for 3 min, to 0:100 over 4 min, maintain at 0:100 for 3 min, to 15:85 over 1 min, stay at 15:85 for 2 min.
Column temperature: 35
Flow rate: 1.5 for 37.5 min, 1 for 10 min
Injection volume: 20
Detector: F ex 338 em 425

CHROMATOGRAM
Retention time: 22.60
Internal standard: homoserine (16.31)
Limit of quantitation: 31 μM

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
plasma; derivatization

REFERENCE
Uhe,A.M.; Collier,G.R.; McLennan,E.A.; Tucker,D.J.; O'Dea,K. Quantitation of tryptophan and other plasma amino acids by automated pre-column o-phthaldialdehyde derivatization high-performance liquid chromatography: improved sample preparation, *J.Chromatogr.*, **1991**, *564*, 81–91.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 5% perchloric acid, centrifuge at 3000 rpm for 10 min. Remove the supernatant and neutralize it with 3 M potassium carbonate, centrifuge at 3000 rpm for 5 min, adjust the volume to 2 mL. (Alternatively, filter (Amicon CF-50) 4 mL plasma while centrifuging at 2500 rpm for 10 min.) 10 μL Perchloric acid extract or ultrafiltrate + 10 μL 100 mM pH 9.0 sodium bicarbonate + 40 μL freshly prepared 4 mM 4-dimethylaminoazobenzene-4'-sulfonyl chloride in MeCN, heat at 70° for 10 min, cool, make up to 500 μL with EtOH:water 70:30, centrifuge at 14000 rpm for 3 min, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-18 T
Column: 150 × 4.6 3 μm Supelcosil LC-18 T
Mobile phase: Gradient. A was 25 mM pH 6.8 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 80:20 for 1 min, to 77:23 over 4 min, maintain at 77:23 for 7 min, to 73:27 over 11 min, to 70:30 over 7 min, to 40:60 over 9 min, to 30:70 over 1 min, maintain at 30:70 for 5 min, return to initial conditions over 1 min, re-equilibrate for 6 min.
Flow rate: 1.5
Injection volume: 5
Detector: UV 436

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
plasma; ultrafiltrate; derivatization

REFERENCE
Stocchi,V.; Palma,F.; Piccoli,G.; Biagarelli,B.; Cucchiarini,L.; Magnani,M. HPLC analysis of taurine in human plasma sample using the DABS-Cl reagent with sensitivity at picomole level, *J.Liq.Chromatogr.*, **1994**, *17*, 347–357.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 200 mg/mL 5-sulfosalicylic acid in EtOH to a 1 mL tube, evaporate EtOH at 50° overnight, add 200-500 μL plasma, vortex, freeze in liquid nitrogen, store at -70°, thaw, centrifuge at 4° at 3000 g. 5 μL Supernatant + 20 μL water + 5 μL 1 mM norvaline in water + 90 μL reagent, mix thoroughly, incubate at room temperature for 3 min, add 50 μL neutralizing buffer, inject a 3 μL aliquot. (Prepare reagent stock solution by dissolving 25 mg o-phthalaldehyde in 500 μL MeOH, add 4.5 mL 100 mM pH 10.0 borate buffer, add 25 μL 3-mercaptopropionic acid. At the start of each day prepare reagent by diluting 1 part of stock solution with 20 parts 100 mM pH 10.0 borate buffer. Neutralizing buffer was 400 mM KH_2PO_4 containing 10 mL/L triethylamine.)

HPLC VARIABLES
Guard column: 10 × 2 Chrompack reverse phase
Column: 100 × 4.6 3 μm Microsphere C18 (Chrompack)
Mobile phase: Gradient. A was buffer:water:THF 50:50:0.2. B was MeOH:MeCN:buffer 35:15:50. A:B from 98:2 to 75:25 over 3.5 min, to 56:44 over 1.7 min, to 48:52 over 1.7 min, to 0:100 over 3.1 min, reset to initial conditions over 1 min.
Flow rate: 1.5
Injection volume: 3
Detector: F ex 230 em 389 (cut-off filter)

CHROMATOGRAM
Retention time: 7.6
Internal standard: norvaline (10.3)
Limit of quantitation: 5000 nM

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
plasma; derivatization

REFERENCE
Teerlink,T.; Van Leeuwen,P.A.M.; Houdijk,A. Plasma amino acids determined by liquid chromatography within 17 minutes, *Clin.Chem.*, **1994**, *40*, 245–249.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 150 μL MeCN, vortex, centrifuge at 5800 g for 10 min. Remove the supernatant and add it to 50 μL buffer, add 50 μL 5 mM fluorescamine in MeCN, vortex, inject an aliquot. (Prepare buffer by adjusting the pH of 100 mM disodium tetraborate to 9.2 with 10 mM boric acid

HPLC VARIABLES
Guard column: C8
Column: 300 × 3.9 10 μm Bondclone C18
Mobile phase: MeCN:THF:buffer 24:4:72 (Prepare buffer by adjusting the pH of 15 mM KH_2PO_4 to 3.5 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 385

CHROMATOGRAM
Limit of quantitation: 5 μg/mL

KEY WORDS
derivatization; plasma

REFERENCE
McMahon,G.P.; O'Kennedy,R.; Kelly,M.T. High-performance liquid chromatographic determination of taurine in human plasma using pre-column extraction and derivatization, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1287–1294.

SAMPLE
Matrix: blood, CSF
Sample preparation: Plasma. For each volume of plasma add 4 volumes of MeOH, centrifuge at 11600 g for 5 min. Remove a 10 μL aliquot and add it to 5 μL phthaldialdehyde/β-mercaptoethanol derivatizing reagent (Fluoraldehyde, Pierce) (use fresh reagent), allow to react at room temperature for 1 min, add 100 μL THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid, inject a 20 μL aliquot. CSF. Add an equal volume of MeOH to the CSF, centrifuge at 11600 g for 5 min. Remove a 10 μL aliquot and add it to 5 μL phthaldialdehyde, allow to react at room temperature for 1 min, add 100 μL THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 Spherisorb 5 ODS
Column: 50 × 4.6 Spherisorb 5 ODS
Mobile phase: Gradient. A was THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid. B was MeOH:THF 95:5. A:B from 10:90 to 0:100 over 13 min (sic), maintain at 0:100 for 4 min, return to initial conditions over 1 min.
Column temperature: 43
Flow rate: 1.5
Injection volume: 20
Detector: F (wavelengths not specified)

CHROMATOGRAM
Retention time: 0.5 (Asp), 0.7 (Glu), 1.2 (Taurine), 1.5 (Ser), 1.8 (Gln), 2.2 (His), 2.3 (Gly), 3.0 (Thr), 3.1 (Asn), 3.2 (Ala), 3.3 (Arg), 3.8 (Tyr), 5.2 (Met), 5.4 (Val), 5.8 (Trp), 6.0 (Phe), 5.5 (Ile), 5.7 (Leu), 8.3 (Orn), 8.5 (Lys)
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, serine, threonine, tryptophan, tyrosine, valine

KEY WORDS
plasma; Thr; Gly; His co-elute; derivatization

REFERENCE
Begley,D.J.; Reichel,A.; Ermisch,A. Simple high-performance liquid chromatographic analysis of free primary amino acid concentrations in rat plasma and cisternal cerebrospinal fluid, *J.Chromatogr.B*, **1994**, *657*, 185–191.

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Prepare a SPE column by placing a 2 cm layer of 200-400 mesh Dowex 2x8 Cl^-form in a column, on top of this place a 2 cm layer of 100-200 mesh Dowex 50 W-x8 H^+form. 1 mL Serum, CSF, or urine + 100 μL 4 M perchloric acid, centrifuge, add the supernatant to the SPE column, elute with three 1 mL portions of water, collect all the effluent from the column. Remove a 1 mL aliquot and add it to 500 μL 1 M pH 9.0 borate buffer, vortex while adding 500 μL 300 μg/mL fluorescamine in MeCN, let stand for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 mm long LiChrosorb RP-18-10A
Mobile phase: MeCN:15 mM pH 6.0 phosphate buffer 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 395 em 455

CHROMATOGRAM
Retention time: 3
Limit of detection: 0.25 pmole

OTHER SUBSTANCES
Noninterfering: amino acids, hypotaurine, phosphoethanolamine

KEY WORDS
serum; derivatization; SPE

REFERENCE
Stabler,T.V.; Siegel,A.L. Rapid liquid-chromatographic/fluorometric method for taurine in biological fluids, involving pre-derivatization with fluorescamine, *Clin.Chem.*, **1981**, *27*, 1771–1771.

SAMPLE
Matrix: blood, food, peptides, plants, tissue
Sample preparation: Hydrolyze peptide with 6 M HCl containing 0.2% 3,3'-thiodipropionic acid at 110° for 24 h, evaporate to dryness, reconstitute with 50-200 μL 0.1% HCl containing 0.2% 3,3'-thiodipropionic acid. Homogenize (Ultra-Turrax) 0.1-1 g food, tissue, plant material, lyophilized plasma, or lyophilized tissue in 10 mL 250 nM IS in 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid at 20000 rpm for 2 min, sonicate for ≤30 min, centrifuge at 5000 g for 20 min, discard fat layer, filter (Millipore ultrafiltration insert (MW cutoff 5000) prewashed with 200 μL 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid) 3 mL supernatant while centrifuging at 3500 g for 1 h. Mix 20 μL deproteinized sample (or 10 μL peptide hydrolysate) with 180 μL buffer, vortex, add 200 μL reagent, mix, heat at 70° for 15 min with mixing at 1 min and 12 min, cool in an ice bath for 5 min, centrifuge at 10000 g for 10 s, add 400 μL diluent, mix thoroughly, centrifuge at 15000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Prepare buffer by dissolving 630 mg sodium bicarbonate in 40 mL water, adjusting pH to 8.6 with NaOH, and making up to 50 mL with water. Prepare reagent by sonicating 40 mg dabsyl chloride in 10 mL acetone for 10 min, then filtering into brown vials and storing at -20°. Prepare diluent by mixing 50 mL MeCN, 25 mL EtOH, and 25 mL mobile phase A.)

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was DMF:9 mM NaH_2PO_4 containing 0.16% triethylamine, adjusted to pH 6.55 with phosphoric acid. B was MeCN:water 80:20. A:B 92:8 for 2 min, to 80:20 over 5 min (Waters convex curve 5), to 65:35 over 28 min (Waters concave curve 7), to 50:50 over 10 min, to 0:100 over 21 min, maintain at 0:100 for 11 min, return to initial conditions over 0.5 min, re-equilibrate for 12.5 min.

Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 436

CHROMATOGRAM
Retention time: 32.31
Internal standard: norleucine (40.90), norvaline (35.06)

OTHER SUBSTANCES
Extracted: amino acids dopamine, epinephrine, histamine, norepinephrine

KEY WORDS
rinse glass and plasticware with 70% EtOH and water and dry before use; derivatization; cheese; meat; sausage; fish; plasma

REFERENCE
Krause,I.; Bockhardt,A.; Neckermann,H.; Henle,T.; Klostermeyer,H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives, *J.Chromatogr.A*, **1995**, *715*, 67–79.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. Mix 9 volumes of plasma with 1 volume of 35% 5-sulfosalicylic acid, centrifuge at 2000 g for 10 min. Neutralize the supernatant with 10 M KOH, dilute with 2 volumes of water. Mix an aliquot with an equal volume of reagent, inject a 20 μL aliquot within 1 min. Tissue. Homogenize tissue with four volumes 5% 5-sulfosalicylic acid, centrifuge at 5000 g for 10 min, neutralize the supernatant with 10 M KOH. Mix an aliquot with an equal volume of reagent, inject a 20 μL aliquot within 1 min. (Prepare reagent each day by dissolving 35 mg o-phthalaldehyde in 500 μL 95% EtOH and adding this mixture to 50 mL 100 mM pH 10.4 borate buffer, add 100 μL 2-mercaptoethanol.)

HPLC VARIABLES
Guard column: 37-50 μm Bondapak C18/Corasil
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was THF:water 3:97 containing 100 mM potassium phosphate, pH 7.0. B was THF:MeCN:water 3:40:57 containing 100 mM potassium phosphate, pH 7.0. A:B 97:3 for 1.5 min, to 68:32 over 17 min (Waters curve profile 3), to 0:100 over 2 min, maintain at 0:100 for 4.5 min, return to initial conditions over 2 min, re-equilibrate for 8 min.
Column temperature: 41
Flow rate: 1
Injection volume: 20
Detector: F ex 360 em 455

CHROMATOGRAM
Retention time: 21.5

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
plasma; human; rat; liver; kidney; heart; brain; derivatization

REFERENCE
Hirschberger,L.L.; De La Rosa,J.; Stipanuk,M.H. Determination of cysteinesulfinate, hypotaurine and taurine in physiological samples by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 303–313.

SAMPLE
Matrix: blood, tissue, urine

Sample preparation: Urine. Adjust urine to pH 7 with 2 M NaOH or 2 M HCl, dilute 5 (human) or 100 (rat) fold with water. Remove a 1 mL aliquot and add 10 μL triethylamine and 20 mg freshly powdered 3,5-dinitrobenzoyl chloride, shake vigorously on a mechanical shaker for 10 min, add 100 μL 2 M HCl, centrifuge at 1200 g for 5 min. Remove a 200 μL aliquot of the supernatant and dilute it to 5 mL with water, inject a 10-100 μL aliquot. Whole blood. Add 7 volumes of water to whole blood, mix, add 1 volume of 10% sodium tungstate dihydrate solution, mix, add with shaking 1 volume of 333 mM sulfuric acid, shake vigorously (J. Biol. Chem. 1919, 38, 81), centrifuge at 1200 g for 10 min. Remove a 1 mL aliquot of the supernatant and add 10 μL triethylamine and 20 mg freshly powdered 3,5-dinitrobenzoyl chloride, shake vigorously on a mechanical shaker for 10 min, add 100 μL 2 M HCl, centrifuge at 1200 g for 5 min. Remove a 200 μL aliquot of the supernatant and dilute it to 5 mL with water, inject a 10-100 μL aliquot. Tissue. Liver or heart + 7 volumes of water + 1 volume 10% sodium tungstate + 1 volume 330 mM sulfuric acid, homogenize (Potter-Elvehjem, glass pestle), centrifuge at 1200 g for 10 min, dilute 2 (liver) or 5 heart) fold with water. Remove a 1 mL aliquot and add 10 μL triethylamine and 20 mg freshly powdered 3,5-dinitrobenzoyl chloride, shake vigorously on a mechanical shaker for 10 min, add 100 μL 2 M HCl, centrifuge at 1200 g for 5 min. Remove a 200 μL aliquot of the supernatant and dilute it to 5 mL with water, inject a 10-100 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 TSK gel ODS-80Ts C18 (Tosoh)
Mobile phase: MeCN:100 mM pH 3.7 ammonium acetate buffer 16:84
Flow rate: 0.8
Injection volume: 10-100
Detector: UV 254

CHROMATOGRAM

Retention time: 18
Limit of detection: 500 nM

OTHER SUBSTANCES

Extracted: amino acids (some), hypotaurine

KEY WORDS

derivatization; human; rat; whole blood; liver; heart

REFERENCE

Masuoka,N.; Yao,K.; Kinuta,M.; Ohta,J.; Wakimoto,M.; Ubuka,T. High-performance liquid chromatographic determination of taurine and hypotaurine using 3,5-dinitrobenzoyl chloride as derivatizing reagent, *J.Chromatogr.B*, **1994**, *660*, 31–35.

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Urine. Centrifuge at 4° at 2500 g for 10 min, add 25-100 μL urine to the SPE column, discard the first 25-100 μL eluate, elute with 4 mL water, add 100 μL 100 μM homoserine to the eluate, add an equal volume of the reagent, let stand for 1.5 min, inject a 5-10 μL aliquot. Liver. Homogenize (polytron) 350-450 mg frozen liver in 4 mL 200 mM sulfosalicylic acid at 4°, centrifuge at 4° at 2500 g for 10 min, add a 25 μL aliquot to the SPE column, elute with 4 mL water, add 100 μL 100 μM homoserine to the eluate, add an equal volume of the reagent, let stand for 1.5 min, inject a 5-10 μL aliquot. Serum. 100 μL Serum + 100 μL 200 mM sulfosalicylic acid, mix at 4°, centrifuge at 4° at 11500 g for 2 min, add a 50 μL aliquot of the supernatant to the SPE column, discard the first 50 μL eluate, elute with 4 mL water, add 100 μL 100 μM homoserine to the eluate, add an equal volume of the reagent, let stand for 1.5 min, inject a 5-10 μL aliquot. Hepatocytes. 500 μL Cell suspension + 500 μL 200 mM sulfosalicylic acid, mix at 4°, centrifuge at 4° at 11500 g for 2 min, add a 200 μL aliquot of the supernatant (or 100 μL of the centrifuged medium) to the SPE column, elute with eight 500 μL aliquots of water, add 100 μL 40 μM homoserine to the eluate, add an equal volume of the reagent, let stand for 1.5 min, inject a 5-10 μL aliquot. (Prepare SPE columns as follows. Wash 100 g 100-200 mesh Dowex 1-X4 (anion-exchange, Cl- form) with three volumes of water to remove fines then with 250 mL 1 M HCl until pH was above 2.5. Wash 100 g 100-200 mesh Dowex 50W-X8 (cation-exhange, H+ form) with three volumes of water to remove fines then with 500 mL 4 M HCl in three washings then with 250 mL 1 M HCl. Prepare a column in a Pasteur pipette with 0.5 mL Dowex 1-X4 on top of 1.5 mL Dowex 50W-X8, wash

with 12 mL water. After use regenerate with 12 mL 1 M HCl. Prepare reagent by adding 40 mg o-phthalaldehyde in 800 μL EtOH and 40 μL 2-mercaptoethanol to 10 mL buffer, dilute the mixture with an equal volume of water. Buffer was 3.1 g boric acid in 90 mL water adjusted to pH 10.4 with 5 M NaOH and made up to 100 mL.)

HPLC VARIABLES
Guard column: 4 × 4 LiChrospher
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water 43:57 containing 50 mM NaH_2PO_4, pH 5.4
Flow rate: 2
Injection volume: 5-10
Detector: F ex 305-395 (filter) em 420-650 (filter)

CHROMATOGRAM
Retention time: 3.7
Internal standard: homoserine (3)
Limit of detection: 0.5 pmole

KEY WORDS
rat; liver; serum; dog; human; SPE; derivatization; hepatocytes

REFERENCE
Waterfield,C.J. Determination of taurine in biological samples and isolated hepatocytes by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1994**, *657*, 37–45.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 10 mL water. 500 μL Plasma + 500 μL 10% trichloroacetic acid, mix, centrifuge at 4° at 9000 g for 10 min. 1 mL Urine + 1 mL 10% trichloroacetic acid, mix, centrifuge at 4° at 9000 g for 10 min. Add a 1 mL aliquot of the supernatant to the SPE cartridge, elute with 2 mL 5% trichloroacetic acid. Collect all the effluent from the SPE cartridge and make up to 10 mL with 5% trichloroacetic acid. Remove a 50 μL aliquot and add it to 150 μL 400 mM pH 9.0 sodium borate buffer, mix, add 50 μL MeOH, add 100 μL 30 mM 7-chloro-4-nitrobenz-2-oxa-1,3-diazole in MeOH, heat at 60° in the dark for 40 min, make up to 1 mL with cold mobile phase, inject a 25-100 μL aliquot.

HPLC VARIABLES
Guard column: CN Guard-PAK (Waters)
Column: 250 × 4 10 μm Partisil SAX
Mobile phase: MeCN:25 mM citric acid 10:90, pH adjusted to 2.9 with 1 M NaOH
Flow rate: 1.3
Injection volume: 25-100
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 10
Limit of detection: 5 pmole

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Palmerini,C.A.; Fini,C.; Cantelmi,M.G.; Floridi,A. Assessment of taurine in plasma and urine by anion-exchange high-performance liquid chromatography with pre-column derivatization, *J.Chromatogr.*, **1987**, *423*, 292–296.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or 50 μL urine + 200 μL water + 200 μL 167 mM sulfuric acid + 300 μL 76 mM sodium tungstate(VI), shake mechanically, let stand for 10 min, centrifuge at 2000 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 50 × 8 Shodex Ionpac C-811P sulfonic acid-type polystyrene porous polymer (Showa Denko, Tokyo)
Column: 300 × 8 Shodex Ionpac KC-811 sulfonic acid-type polystyrene porous polymer (Showa Denko, Tokyo)
Mobile phase: 10 mM Phosphoric acid
Column temperature: 45
Flow rate: 0.8
Injection volume: 20
Detector: F ex 370 em 440 following post-column reaction. The column effluent mixed with the oxidant pumped at 0.3 mL/min and this mixture flowed through a 3 m × 0.5 mm ID PTFE coil at 65°. The effluent from this coil mixed with the reagent pumped at 0.3 mL/min and this mixture flowed through a 5 m × 0.5 mm ID PTFE coil at 65° and a 1 m × 0.5 mm ID PTFE cooling coil to the detector. (Prepare oxidant by adding 60 mL 1 M NaOH and 4 mL 25% Brij-35 to 6 mL 10% sodium hypochlorite solution, make up to 1 L with 100 mM pH 7.0 phosphate buffer, final pH 12.0. Prepare reagent by dissolving 22.8 g sodium nitrite and 200 mg thiamine hydrochloride in 100 mM pH 7.0 phosphate buffer, adjust to pH 7.0 with 100 mM Na_2HPO_4 and 100 mM NaH_2PO_4, make up to 1 L with 100 mM pH 7.0 phosphate buffer. Thiamine is oxidized to the fluorescent thiochrome.)

CHROMATOGRAM

Retention time: 14
Limit of detection: 6 ng

KEY WORDS

post-column reaction; serum

REFERENCE

Yokoyama,T.; Kinoshita,T. High-performance liquid chromatographic determination of taurine in biological fluids by post-column fluorescence reaction with thiamine, *J.Chromatogr.*, **1991**, *568*, 212–218.

SAMPLE

Matrix: formula, media, tissue
Sample preparation: Boil 500 g tuna or squid meat in 1 L water for 2 h, centrifuge an aliquot at 15000 g for 3 min. Dilute 1 g infant formula with 1 mL water, centrifuge at 15000 g for 3 min. Centrifuge an aliquot of liquid media containing bacteria at 15000 g for 3 min. 100 μL Supernatant + 100 μL 4% NaOH, mix, add 20 μL 10% 2,4-dinitrofluorobenzene in acetone, shake vigorously, let stand for 15 min, add 100 μL 10% orthophosphoric acid, add 500 μL chloroform, shake, centrifuge at 15000 g for 15 s. Remove 200 μL of the upper aqueous layer, add 500 μL chloroform, shake, centrifuge at 15000 g for 15 s, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Column: 50 × 4 10 μm Silosorb C18 (Elsico, Moscow)
Mobile phase: MeOH:water:glacial acetic acid:triethylamine 25:75:1:0.1
Flow rate: 5
Injection volume: 1
Detector: UV 350

CHROMATOGRAM

Retention time: 0.2
Limit of detection: 10 pmole

OTHER SUBSTANCES

Extracted: cysteic acid

KEY WORDS

fish; tuna; squid; derivatization

REFERENCE

Polanuer,B.; Ivanov,S.; Sholin,A. Rapid assay of dinitrophenyl derivative of taurine by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 81–85.

SAMPLE
Matrix: formula, milk
Sample preparation: 3 g Milk or formula + 80 mL water, heat at 50-60° with periodic agitation for 10 min, cool to room temperature, add 1 mL 150 mg/mL potassium ferrocyanide trihydrate in water, swirl, add 1 mL 300 mg/mL zinc acetate dihydrate in water, mix, let stand with periodic inversion for 20 min, make up to 100 mL with water, mix thoroughly, filter, discard the first 3-5 mL. Mix 1 mL filtrate with 1 mL buffer, add 1 mL 1.5 mg/mL dansyl chloride in MeCN, mix by inversion, let stand in the dark (with mixing after 1 h) at room temperature for 2 h, add 100 μL 20 mg/mL methylamine hydrochloride in water, vortex, filter (0.45 μm), inject a 20 μL aliquot of the filtrate. (Buffer was 80 mM sodium carbonate adjusted to pH 9.5 with 1 M HCl.)

HPLC VARIABLES
Guard column: C18
Column: 5 μm Resolve (Waters)
Mobile phase: MeCN:buffer 16:84
Flow rate: 1
Injection volume: 20
Detector: UV 254, F ex 330 em 530

CHROMATOGRAM
Retention time: 4-6
Limit of detection: 50 μg/g

KEY WORDS
derivatization

REFERENCE
Woollard,D.C.; Indyk,H.E. Taurine analysis in milk and infant formulae by liquid chromatography: Collaborative study, *J.AOAC Int.*, **1997**, *80*, 860–865.

SAMPLE
Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeCN:MeOH:139 mM Na_2HPO_4 3.1:25:71.9 adjusted to pH 6.8 with phosphoric acid
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 450 mV

CHROMATOGRAM
Retention time: 18.99
Limit of detection: 0.75 ng/mL

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
rat; pharmacokinetics; derivatization

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: solutions
Sample preparation: Mix sample:50 (?) mM NaCN in 50 mM pH 9.3 borate buffer:25 (?) mM naphthalene-2,3-dicarboxaldehyde in MeOH 3:1:1, let stand for 15 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 3 5 μm Chromspher ODS-2 C18 (Chrompack)
Mobile phase: Gradient. A was 50 mM pH 7.0 sodium phosphate buffer. B was MeOH:THF:water 50:20:30. A:B from 25:75 to 0:100 over 75 min.
Flow rate: 0.5
Injection volume: 50
Detector: F ex 420

CHROMATOGRAM
Retention time: 40

OTHER SUBSTANCES
Simultaneous: amino acids

KEY WORDS
derivatization

REFERENCE
Koning,H.; Wolf,H.; Venema,K.; Korf,J. Automated precolumn derivatization of amino acids, small peptides, brain amines and drugs with primary amino groups for reversed-phase high-performance liquid chromatography using naphthalenedialdehyde as the fluorogenic label, *J.Chromatogr.*, **1990**, *533*, 171–178.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Kontes micro-ultrasonic cell disrupter) 1-5 mg rat pineal tissue in 200 μL water, add 200 μL 2% picric acid in water (Caution! Picric acid is toxic and explosive when dry!), let stand at room temperature for 5 min, add to the column, rinse the container with three 200 μL portions of water, add the rinses to the column, add 1 mL water to the column, lyophilize the eluate, reconstitute with 100 μL water. Mix an aliquot of this solution with an equal volume of the reagent, let stand for 1 min, inject a 2-6 μL aliquot. (Prepare the column by making a 25 mm layer of Bio-Rad 200-400 mesh 50W-X8 (hydrogen form)ion-exchange resin in a 5 mm diameter column, add a 25 mm layer of 100-200 mesh Bio-Rad AG 1-X8 (chloride form) ion-exchange resin on top of this, wash with 10 mL water just before use. Prepare the reagent by dissolving 20 mg o-phthalaldehyde in 400 μL EtOH, adding 20 μL 2-mercaptoethanol, and adding 10 mL 500 mM pH 10.3 borate buffer. Dilute 1:10 with water before use.)

HPLC VARIABLES
Column: 300 × 3.9 μBondapak alkyl phenyl
Mobile phase: MeOH:buffer 42.75:57.25 (Prepare by mixing A:B in the ratio 43:57. A was prepared by dissolving 6.9 g $NaH_2PO_4.H_2O$ in water, adjusting pH to 5.3 with 5 M NaOH, and making up to 1 L with water. B was prepared by dissolving 6.9 g $NaH_2PO_4.H_2O$ in 250 mL water and making up to 1 L with MeOH.)
Flow rate: 2
Injection volume: 2-6
Detector: F ex 360 em 455

CHROMATOGRAM
Retention time: 4
Limit of quantitation: 600 pg

OTHER SUBSTANCES
Noninterfering: cysteic acid, hypotaurine, phosphoethanolamine

KEY WORDS
rat; pineal; SPE; derivatization

REFERENCE
Larsen,B.R.; Grosso,D.S.; Chang,S.Y. A rapid method for taurine quantitation using high performance liquid chromatography, *J.Chromatogr.Sci.*, **1980**, *18*, 233–236.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron for >10 mg; Kontes micro-ultrasonic cell disrupter for <10 mg) tissue with 40 volumes 25 μg/mL β-aminoisobutyric acid in EtOH:water:glacial acetic acid 75:20:5, centrifuge at 4° at 25000 g for 20 min. Remove a 50 μL aliquot of the supernatant and evaporate it to dryness under reduced pressure, suspend the residue in 100 μL 100 mM sodium bicarbonate by sonicating or vortexing, add 200 μL 1.25 mg/mL dansyl chloride in acetone, vortex, heat at 90° for 30 min, centrifuge at 5000 g for 20 min, inject a 4 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:water:phosphoric acid 13:87:0.15
Flow rate: 1
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.26
Internal standard: β-aminoisobutyric acid (k' 9.25)
Limit of quantitation: 10 pmole

OTHER SUBSTANCES
Extracted: amino acids, urea
Interfering: asparagine, methionine

KEY WORDS
rat; brain; derivatization

REFERENCE
Saller,C.F.; Czupryna,M.J. γ-Aminobutyric acid, glutamate, glycine and taurine analysis using reversed-phase high-performance liquid chromatography and ultraviolet detection of dansyl chloride derivatives, *J.Chromatogr.*, **1989**, *487*, 167–172.

SAMPLE
Matrix: tissue, dialysate
Sample preparation: Homogenize (Kontes micro-ultrasonic cell disrupter) rat brain with 100 μL 50 mM ice-cold perchloric acid and 10 ng homoserine for 5 s, centrifuge at 4° at 13000 g for 5 min, filter (0.2 μm) the supernatant. Mix 25 μL of the filtrate from the tissue or dialysate (Ringer's) with 50 (tissue) or 12.5 (dialysate) μL working reagent, let stand for 2 min, inject an aliquot. (Prepare the reagent stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate, discard after 5 days. Prepare the working reagent by diluting 1 mL stock solution with 3 mL 100 mM sodium tetraborate, let stand for 24 h before use.)

HPLC VARIABLES
Column: 80 × 4.6 3 μm C18 HR-80 (ESA)
Mobile phase: MeOH:water 28:72 containing 100 mM Na_2HPO_4 and 0.13 mM disodium EDTA adjusted to pH 6.00 (tissue) or pH 6.40 (dialysate) with phosphoric acid. (Prepare by dissolving 14.2 g Na_2HPO_4 and 50 mg disodium EDTA in 720 mL water, add 280 mL MeOH, adjust pH. Recycle mobile phase.)

Flow rate: 1.2
Injection volume: 20
Detector: E, ESA Model 5100A coulometric, model 5011 dual electrode analytical cell preceded by a 0.2 μm carbon filter at -0.4 V and +0.6 V

CHROMATOGRAM
Retention time: 9
Internal standard: homoserine (3.5)
Limit of detection: 100-200 pg

OTHER SUBSTANCES
Extracted: amino acids

KEY WORDS
rat; brain; derivatization

REFERENCE
Donzanti,B.A.; Yamamoto,B.K. An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates, *Life Sci.*, **1988**, *43*, 913–922.

SAMPLE
Matrix: urine
Sample preparation: 10 μL Urine + 50 μL 5 mM S-carboxymethyl-L-cysteine in water + 500 μL 100 mM pH 9.0 sodium bicarbonate buffer + 50 μL acetone + 1 mL 650 μg/mL dabsyl chloride in acetone, heat at 40° for 30 min, add 1.5 mL EtOH, let stand for 30 min, centrifuge, filter (0.2 μm) the supernatant, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 15 × 3.2 5 μm TSKguardgel ODS-80Ts
Column: 150 × 4.6 5 μm TSKgel ODS-80Ts
Mobile phase: Gradient. MeCN:50 mM pH 4.00 sodium acetate buffer from 28:72 to 31:69 over 20 min, to 32:68 over 20 min, wash with 95:5 for 5 min, re-equilibrate at initial conditions for 20 min.
Column temperature: 16
Injection volume: 10
Detector: UV 430

CHROMATOGRAM
Retention time: 27
Internal standard: S-carboxymethyl-L-cysteine (23)
Limit of detection: 4 pmole

OTHER SUBSTANCES
Extracted: hypotaurine

KEY WORDS
rat; derivatization

REFERENCE
Futani,S.; Ubuka,T.; Abe,T. High-performance liquid chromatographic determination of hypotaurine and taurine after conversion to 4-dimethylaminoazobenzene-4'-sulfonyl derivatives and its application to the urine of cysteine-administered rats, *J.Chromatogr.B*, **1994**, *660*, 164–169.